FINAL TECHNICAL REPORT

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PRINCIPAL INVESTIGATOR: Dr. John Lee Spudich

INSTITUTION: The University of Texas Medical School

GRANT TITLE: Role of Protein Methylation in *Halobacterium halobium* Phototaxis

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OBJECTIVE: To investigate the role of methyl-accepting proteins in the phototaxis signaling system of *H. halobium* membranes. A carboxylmethylated protein in the membrane, HtrI (halobacterial transducer for sensory rhodopsin I) appears to relay the signal from photoactivated sensory rhodopsin I (SR-I, a visual pigment-like photosensor). Our primary objective was to elucidate the relationship between SR-I and HtrI.

APPROACH: HtrI primary structure and other properties were determined by purification of the protein, tryptic digestion and isolation of fragments for peptide sequencing, and use of sequence-derived oligonucleotide probes to clone the HtrI-encoding gene. The *htrI* gene was cloned, mapped, and sequenced. It was found to be in an operon-like arrangement with the SR-I-encoding *sopI* gene. A fragment containing the *htrI-sopI* region was used to transform *H. halobium* and restore phototaxis function to a mutant in which the region is deleted.

ACCOMPLISHMENTS: In earlier work on this project a methylated membrane protein of 97kDa M_r was suggested on the basis of mutant analysis to transduce signals from the phototaxis receptor sensory rhodopsin I to the flagellar motor in *H. halobium* (Spudich et al, Proc. Natl. Acad. Sci. USA 86:7746-7750, 1989). In this period we completed the cloning of the proposed transducer protein gene based on partial protein sequences from the isolated protein, the complete gene sequence, analysis of the encoded primary structure, and expression of the gene. The gene ends immediately at the initiator codon of the *sopI* gene which encodes the sensory rhodopsin I apoprotein. Comparison of the translated nucleotide sequence with N-terminal sequence of the purified protein shows the protein is synthesized without a processed leader peptide and the N-terminal methionine is removed in the mature protein. The deduced protein sequence predicts two transmembrane helices near the N-terminal which would anchor the protein to the membrane. Beyond this hydrophobic region of 46 residues, the remainder of the protein (535 amino acid residues total) is hydrophilic. The C-terminal 270 residues contain a region homologous to the signalling domains of

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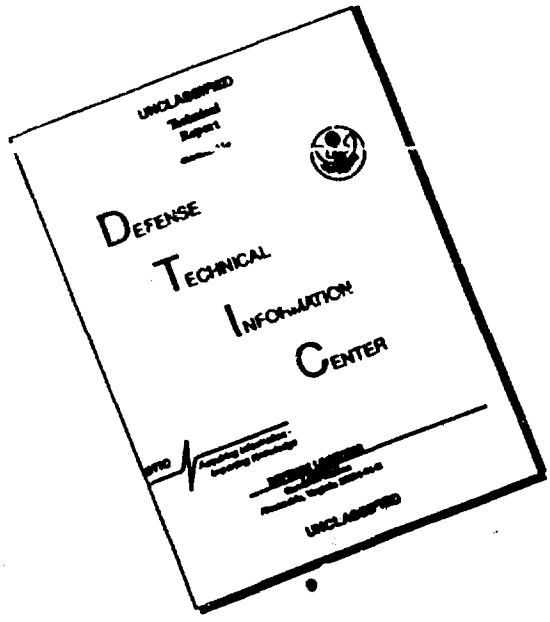

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eubacterial transducers (e.g. *Eschericbia coli* Tsr protein), flanked by two regions homologous to the methylation domains of the transducer family. The predicted protein structure differs from that of *E. coli* Tsr in that it does not have an extramembraneous receptor binding domain, but instead has a more extended cytoplasmic region.

SIGNIFICANCE: These results (1) extend the eubacterial transducer family to the archaebacteria; (2) substantiate the proposal that the methylated membrane protein functions as a signal transducing relay between SR-I and cytoplasmic sensory pathway components, and (3) provide the molecular genetic tools for study of receptor/transducer interaction.

PUBLICATIONS since October 1, 1991:

1. Yan, B., Nakanishi, K. and Spudich, J. L. (1991) Mechanism of activation of sensory rhodopsin - I: Evidence for a steric trigger. Proc. Natl. Acad. Sci. USA 88:9412-9416.

2. Yan, B. and Spudich, J. L. (1991) Evidence the repellent receptor form of sensory rhodopsin I is an attractant signaling state. Photochem. Photobiol. 54:1023-1026.

3. Yan, B., Takahashi, T., and Spudich, J. L. (1991) Identification of signaling states of a sensory receptor by modulation of lifetimes of stimulus-induced conformations: The case of sensory rhodopsin II. Biochemistry 30:10686-10692.

4. Yan, B., Cline, S. W., Doolittle, W. F., and Spudich, J. L. (1992) Transformation of a BOP$^-$HOP$^-$SOP-I$^-$SOP-II$^-$ *Halobacterium halobium* mutant to BOP$^+$: Effects of bacteriorhodopsin photoactivation on cellular proton fluxes and swimming behavior. Photochem. Photobiol. 56:553-561.

5. Spudich, J. L., and Bogomolni, R. A. (1992) Sensory rhodopsin I: Receptor activation and signal relay. Biomemb. and Bioenerg. 24:193-199.

6. Olson, K. D., Deval, P. and Spudich, J. L. (1992) Absorption and photochemistry ot sensory rhodopsin-I: pH effects. Photochem. Photobiol. 56:1181-1187.

7. Takahashi, T., Yan, B. and Spudich, J. L. (1992) Sensitivity increase in the photophobic response of *Halobacterium halobium* reconstituted with retinal analogs: a novel interpretation for the fluence-response relationship and a kinetic model. Photochem. Photobiol. 56:1119-1128.

8. Yao, V. J. and Spudich, J. L. (1992) Primary structure of an archaebacterial transducer, a methyl-accepting protein associated with sensory rhodopsin I. Proc. Natl. Acad. Sci. USA 89:11915-11919.

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10. Krebs, M. P., Spudich, E. N., Khorana, H. G. and Spudich, J. L. (1993) Synthesis of a gene for sensory rhodopsin I and its functional expression in *Halobacterium halobium*. Proc. Natl. Acad. Sci. USA 90:3486-3490.

Related papers in this period:

1. Khan, S., Amoyaw, K., Spudich, J. L., Reid, G. P. and Trentham, D. R. (1992) Bacterial chemoreceptor signaling probed by flash photorelease of a caged serine. Biophysical J. 62:67-68.

2. Lawson, M. A., Zacks, D. N., Derguini, F., Nakanishi, K., and Spudich, J. L. (1991) Retinal analog restoration of photophobic responses in a blind *Chlamydomonas reinhardtii* mutant: Evidence for an archaebacterial-like chromophore in a eukaryotic rhodopsin. Biophys. J. 60:1490-1498.

3. Zacks, D. N., Derguini, F., Nakanishi, K. and Spudich, J. L. (1993) Comparative study of phototactic and photophobic receptor chromophore properties in *Chlamydomonas reinhardtii*. Biophysical J. *in press*.